Toxicity Assessment of Ethanol Extract from *Castanopsis Costata* (Blume.) A. DC Leaves on the Microscopic Structure of White Rat Kidneys

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Track Record Article

Revised: 26 May 2025
Accepted: 25 November 2025
Published: 31 December 2025

How to cite :
Nasution, P. R., & Zulfikri. (2025). Toxicity Assessment of Ethanol Extract from Castanopsis Costata (Blume.) A. DC Leaves on the Microscopic Structure of White Rat Kidneys. *Contagion : Scientific Periodical of Public Health and Coastal Health*, 7(3), 26–33.

Abstract

Castanopsis Costata (Blume.) A. DC. leaves are traditionally used for medicinal purposes and are known to be rich in bioactive compounds such as flavonoids, tannins, and alkaloids. However, scientific validation regarding their safety profile remains limited. This study aimed to evaluate the acute toxicity effects of ethanol extract from Castanopsis Costata leaves on the renal histopathology of white rats (Rattus norvegicus). The study used a pretest and posttest in the experimental design method. Twenty-five male rats were randomly assigned into five groups and orally administered the ethanol extract at doses of 5, 50, 300, 2000, and 5000 mg/kg body weight for seven consecutive days. On the eighth day, the rats were euthanized, and kidney tissues were collected for microscopic examination. Data normality and homogeneity were assessed before conducting one-way ANOVA followed by Tukey's HSD post hoc test. Histopathological evaluation revealed no significant morphological alterations in renal tissues across all treatment groups. Statistical analysis confirmed that the data were normally distributed and homogeneous ($p > 0.05$). One-way ANOVA indicated no significant nephrotoxic effects associated with extract administration across the tested doses ($p = 0.023$). The ethanol extract of Castanopsis Costata leaves exhibited no observable renal toxicity at doses up to 5000 mg/kg body weight in white rats, suggesting its potential safety for further pharmacological exploration. Future studies should assess sub chronic and chronic toxicity to establish a more comprehensive safety profile

Keywords: Castanopsis Costata, Ethanol Extract, Renal Histopathology, Acute Toxicity, White Rats

INTRODUCTION

Indonesia is rich with plants that can be used for medicines, and this creates opportunities for developing herbal-based products in pharmaceutical industries, for examples, Jamu (traditional drink made of herbs), herbal medicines, and Phytopharmaceuticals (Dewantari et al., 2018).

Indonesia is the largest exporter of herbal medicines because there are more than 30,000 species of plants and animals in Indonesia, and approximately 9,600 of them can be used for medicinal purposes. (BPOM RI, 2023). One of them is the leaves of *Castanopsis Costata* (Blume.) A. DC., or what is known as "*cep cepan*". This plant is often used to treat various diseases, especially in healing wounds, because of its antioxidant and antibacterial. (Alkandahri et al., 2023).

Flavonoid and polyphenolic compounds are known for their antioxidant, anti-inflammatory, and antidiabetic activities. Meanwhile, alkaloid compounds have demonstrated

potential in inhibiting cancer cell proliferation (Putri et al., 2023). Furthermore, decoctions of *Castanopsis Costata* leaves are used for diabetic white rats have been shown to reduce blood glucose levels (Alkandahri et al., 2022; Nangoy et al., 2019; Brata et al., 2022).

Previous studies have evaluated the antimicrobial activity of *Castanopsis Costata* leaves, demonstrating positive effects against Propionibacterium acnes. In addition, community outreach programs have also introduced the use of *Castanopsis Costata* leaves as an adjuvant therapy for Diabetes Mellitus. Furthermore, analgesic activity testing revealed that a dose of 250 mg/kgBW of the leaf extract exhibited an analgesic effect comparable to that of the 500 mg/kgBW extract and 65 mg/kgBW of the standard analgesic agent, antalgin (Syahputra et al., 2022; Alkandahri e.t al., 2022; Salim et al., 2017).

To be used for clinical practice, medicinal plants must be proven save and pharmacologically tested, which is usually achieved through toxicity testing to see the structural and functional alterations in critical organs (liver and kidneys) (Diina et al., 2022).

With such benefits, the safety profile of *Castanopsis Costata* leaves has not been previously evaluated; thus, toxicity testing is essential. The toxicity testing can determine the spectrum of toxic effects and the dose-toxicity relationship following repeated administration over a specified period. Typically, toxicity assessments are conducted in vivo using experimental animals. Based on these considerations, the present study aims to evaluate the safety of ethanol extract from *Castanopsis Costata* leaves to ensure its benefits and evidence-based application.

METHODS

This experimental study employed a one-group pretest-posttest design to evaluate the toxicity effects of ethanol extract from *Castanopsis Costata* (Blume.) A. DC. leaves on the kidneys of white rats (*Rattus norvegicus*). The study was conducted from May to December 2024 at the Pharmacology Laboratory of the Politeknik Kesehatan Kementerian Kesehatan Medan, with an ethical approval obtained from the Research and Community Service Center (P3M) of Poltekkes Kemenkes Medan. All procedures followed ethical standards for the use of experimental animals.

A total of 25 healthy male rats aged 2–3 months and weighing 200–300 grams were randomly assigned into five treatment groups (n=5 per group). The negative control group received 0.5% NaCMC orally, while the treatment groups received oral doses of *Castanopsis Costata* ethanol extract at 5 mg/kgBW, 50 mg/kgBW, 300 mg/kgBW, 2000 mg/kgBW, and 5000 mg/kgBW, respectively, for seven consecutive days.

The *Castanopsis Costata* leaves were collected through purposive sampling from Langkat, North Sumatra. The leaves were shade-dried under good air circulation to preserve their phytochemical content, ground into powder, and extracted using the maceration method with 96% ethanol, and the resulting extract was concentrated using a rotary evaporator. Phytochemical screening confirmed the presence of secondary metabolites, including alkaloids, flavonoids, tannins, and steroids.

On the eighth day, all rats were humanely euthanized, and a necropsy was performed to collect kidney tissues for histopathological examination. Tissue specimens were microscopically assessed for morphological changes associated with extract administration.

Data were analyzed using SPSS version 25.0 for Windows, and the Shapiro-Wilk test was employed to assess the normality of data distribution, followed by the homogeneity of variance test. One-way analysis of variance (ANOVA) was performed to detect statistically significant differences among groups in that significant differences were found ($p<0.05$), and Tukey's Honest Significant Difference (HSD) post hoc test was applied to further explore intergroup differences. All statistical analyses were conducted with a 95% confidence level.

RESULT

The plant material used in this study was identified at the Herbarium Medanese, University of Sumatera Utara (USU), and verified as *Castanopsis Costata* (Blume.) A. DC., based on certificate number 1265/MEDA/2023, and the authenticity of the sample for research purposes was confirmed.

A total of 5 kg of *Castanopsis Costata* leaves were gathered from Langkat, sorted to remove extraneous materials, and washed under running water to eliminate impurities. The leaves were then chopped, oven-dried, and subsequently ground into a fine powder using a blender. Extraction was performed using the maceration method with 96% ethanol as the solvent, following the recommendation of Lloyd et al. (1997), as ethanol is considered a universal solvent. A total of 500 grams of leaf powder was macerated with 4.6 liters of 96% ethanol for 24 hours. The residue was further macerated with an additional 1.5 liters of ethanol. The resulting filtrates were concentrated using a rotary evaporator to obtain a thick extract. A final yield of 312 grams of thick extract was obtained, corresponding to an extraction yield of 20.18%.

Tabel 1. Phytochemical Screening Results of Castanopsis Costata Leaf Extract

Phytochemical Compounds	Detection Result
Alkaloids	(+)
Saponins	(-)
Tannins	(+)
Flavanoids	(+)
Steroids	(+)

Phytochemical screening of the *Castanopsis Costata* leaf extract revealed the presence of alkaloids, tannins, flavonoids, saponins, and steroids.

Tabel 2. Mean Percentage Reduction in Blood Glucose Levels

Group	Mean Reduction in Blood Glucose Level (mg/dL)
Na CMC 1%	173,4
Glibenclamide 5 mg/70 kg BW	83
EEDCC 50 mg/kg BW	117,8
EEDCC 100 mg/kg BW	129,6
EEDC 200 mg/kg BW	148,4

Blood glucose levels were measured in Balb/C mice aged 2–3 months. Initial blood glucose measurements were taken before treatment. The mice were then induced with streptozotocin (STZ) at a dose of 40 mg/kg body weight via intraperitoneal injection and left for three days. On the third day, blood glucose levels were measured again and compared to the baseline values. Mice with blood glucose levels exceeding 200 mg/dL were selected for further experimentation. The selected diabetic mice were subsequently administered ethanol extract of *Castanopsis Costata* at doses of 50 mg/kgBW, 100 mg/kgBW, and 200 mg/kgBW for 12 days. Blood glucose measurements were performed every three days during the treatment period.

Table 3. Normality Test Results

Variable	Kolmogorov-Smirnov			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Blood Glucose Level (Baseline)	0.115	25	0.2	0.964	25	0.502
Blood Glucose Level (Post-Treatment)	0.164	25	0.08	0.951	25	0.268

p > 0.05 indicates that the data are normally distributed

The Kolmogorov-Smirnov normality test indicated that the data were normally distributed, with a significance value of 0.200. This result confirms that the overall data followed a normal distribution. A homogeneity test was subsequently conducted to assess the equality of variances in the percentage reduction of blood glucose levels across the five treatment groups. The homogeneity test yielded a significance value of 0.200 (> 0.05),

indicating no significant differences in variance among the groups. Therefore, the data were considered homogeneous.

Based on the results of the normality and homogeneity tests, the percentage reduction in blood glucose levels was confirmed to be normally distributed and homogeneous, fulfilling the assumptions required for analysis of variance (ANOVA). The ANOVA test results are presented below.

Table 4. ANOVA Test Results on Post-Treatment Blood Glucose Levels

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5378.16	4	1344.54	3.581	0.023
Within Groups	7508.4	20	375.42		
Total	12886.56	24			

Significant differences were observed among the treatment groups (p < 0.05)

The results of the ANOVA test revealed a significant difference in the mean percentage reduction of blood glucose levels among the treatment groups, with a probability value of $p < 0.05$. Specifically, the analysis of post-treatment blood glucose levels across groups yielded an F-value of 3.581 ($F(4, 20) = 3.581$) with a significance level of $p = 0.023$. Following the ANOVA, a post hoc analysis using Tukey's Honest Significant Difference (HSD) test was performed, and the results are presented as follows.

Tabel 5. Post Hoc Tukey HSD Test Results

(I) Treatment Groups		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
KP	KN	-5,200	12,254	0,993	-41,87	31,47
	E50	34,200	12,254	0,075	-2,47	70,87
	E100	24,000	12,254	0,321	-12,67	60,67
	E200	15,800	12,254	0,700	-20,87	52,47
KN	KP	5,200	12,254	0,993	-31,47	41,87
	E50	39.400*	12,254	0,031	2,73	76,07
	E100	29,200	12,254	0,161	-7,47	65,87
	E200	21,000	12,254	0,448	-15,67	57,67
E50	KP	-34,200	12,254	0,075	-70,87	2,47
	KN	-39.400*	12,254	0,031	-76,07	-2,73
	E100	-10,200	12,254	0,917	-46,87	26,47
	E200	-18,400	12,254	0,573	-55,07	18,27
E100	KP	-24,000	12,254	0,321	-60,67	12,67
	KN	-29,200	12,254	0,161	-65,87	7,47
	E50	10,200	12,254	0,917	-26,47	46,87
	E200	-8,200	12,254	0,961	-44,87	28,47
E200	KP	-15,800	12,254	0,700	-52,47	20,87
	KN	-21,000	12,254	0,448	-57,67	15,67
	E50	18,400	12,254	0,573	-18,27	55,07
	E100	8,200	12,254	0,961	-28,47	44,87

**** The mean difference is significant at the 0.05 level***

Tukey's Honest Significant Difference (HSD) test was used to determine the differences between groups. The results, at a 95% confidence level, indicated a significant difference between the positive treatment group and the negative control group.

Table 6. Paired t-Test Results

		Standardizer[a]	Point Estimate	95% Confidence Interval	
				Lower	Upper
Blood Glucose Level Before Induction – Blood Glucose Level After Treatment	Cohen's d	37,651	3,464	2,409	4,508
	Hedges' correction	38,252	3,410	2,371	4,437

The paired t-test results demonstrated a significant difference in blood glucose levels between the induction phase and post-treatment across all groups.

DISCUSSION

The findings indicate that oral administration of ethanol extract from *Castanopsis Costata* leaves at escalating doses of 5 mg/kgBW, 50 mg/kgBW, 300 mg/kgBW, 2000 mg/kgBW, and 5000 mg/kgBW given for seven consecutive days did not cause significant morphological alterations in the kidney tissues of white rats (*Rattus norvegicus*). Histopathological examination revealed intact glomerular and tubular structures which means the extract did not induce nephrotoxic effects within the tested dose range.

These findings are consistent with previous studies in that plant extracts rich in flavonoids, tannins, and alkaloids possess protective potential against renal tissue damage induced by oxidative stress. Flavonoids are well known for their antioxidant activitywhich can protect kidney tissues from free radical-induced injury (Peng et al., 2023). The secondary metabolites present in *Castanopsis Costata* leaves are presumed to maintain the integrity of renal cellular structures through mechanisms involving free radical scavenging and membrane stabilization.

This activity is crucial in warding off free radicals, which are the main triggers of renal tissue injury (Alkandahri et al., 2024). This protective mechanism involves maintaining the integrity of cellular membranes and preventing oxidative damage to kidney cells, and this explains why nephrotoxicity does not occur (Rousdy et al., 2024). This is in line with studies by Mayanti et al., (2020), who found that ethanol extracts from plants with high flavonoid content did not induce nephrotoxicity after repeated administration.

This activity strengthens the basis of traditional use *C. costata* (Alkandahri et al., 2024). and it supports the role of flavonoids as antidiabetic agents (Tamahiwu et al., 2023). With its proven efficacy potential and high acute safety, this extract holds promise as a

phytopharmaceutical candidate. Overall, these findings provide a strong scientific basis to support the safety of *Castanopsis Costata* leaf ethanol extract on kidney tissue in the context of acute use, so it is safe to proceed to the long-term toxicity study phase.

Moreover, the absence of histopathological alterations even at high doses up to 5000 mg/kgBW suggests that the extract possesses a wide safety margin for acute use, and this is consistent with the OECD guidelines (2001) in that substances with no observed adverse effects at doses exceeding 2000 mg/kgBW can be classified as having low acute toxicity potential.

A similar study conducted by Mayanti et al., (2020), who also found that ethanol extracts of plants with high flavonoid content did not induce nephrotoxicity after repeated administration. However, the present study only evaluated acute exposure over a short duration (7 days), thus, cumulative toxic effects resulting from subchronic or chronic exposure cannot be fully concluded from short-term studies (Rahmatudina et al., 2023).

Although the present findings provide preliminary evidence that the ethanol extract of *Castanopsis Costata* leaves is safe for renal tissues in the short term, further research is necessary to evaluate its subchronic and chronic toxicity. Future studies should include biochemical analyses of renal function parameters, such as serum creatinine and blood urea nitrogen levels. Additionally, assessments of other vital organs, such as the liver and heart, are recommended to establish a more comprehensive safety profile.

CONCLUSION

This study concludes that oral administration of *Castanopsis Costata* (Blume) DC leaf extract at doses of 5 mg/kgBW, 50 mg/kgBW, 300 mg/kgBW, 2000 mg/kgBW, and 5000 mg/kgBW for seven days resulted in normal microscopic kidney morphology in white rats. These findings indicate that the ethanol extract of *Castanopsis Costata* leaves is relatively safe for renal tissues within the tested dose range and duration. To strengthen the evidence of its safety, further studies are recommended to conduct subchronic and chronic toxicity evaluations with extended administration periods, including biochemical assessments of renal and hepatic function. Additionally, histopathological analyses of other vital organs, such as the liver and heart, as well as evaluations of oxidative stress biomarkers, are highly encouraged to establish a more comprehensive safety profile before its development as a potential phytopharmaceutical candidate.

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